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IL-17 Induces Inflammation-Associated Gene Products in Blood Monocytes, and Treatment with Ixekizumab Reduces Their Expression in Psoriasis Patient Blood

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TO THE EDITOR

Recently, our understanding of psoriasis has expanded from a superficial skin disease to a systemic inflammatory process that affects multiple organs. It has been postulated that circulating high levels of pro-inflammatory cytokines may predispose psoriasis patients to serious comorbid conditions such as atherosclerosis, psoriatic arthritis, obesity, and diabetes (Davidovici *et al.*, 2010). However, the mechanistic link between increased circulating cytokine levels and these comorbid conditions remains to be established.

IL-17A (IL-17) is known to be a central cytokine in psoriasis pathogenesis. We previously reported significant clinical improvements in patients treated with ixekizumab (anti-IL-17A monoclonal antibody), which were accompanied by a rapid reversal of epidermal hyperplasia and dermal infiltration of leukocytes (Krueger *et al.*, 2012). It is known that the level of IL-17 is elevated in the blood of psoriasis patients (Suárez-Fariñas *et al.*, 2012) but the effects of IL-17 on circulating leukocytes bearing IL-17 receptors are not fully understood. Human monocytes highly express both IL-17RA and IL-17RC, and chemotraffic in response to IL-17 (Shahrara *et al.*, 2009), and the activation and recruitment of effector monocytes are known to have a role in the pathobiology of a number of systemic inflammatory diseases, including comorbidities associated with psoriasis, such as atherosclerosis, metabolic regulation in adipose tissue, and psoriatic arthritis. In this study we explored the role of IL-17 in regulating monocyte activities in culture as well as its impact on systemic inflammation in psoriasis patients treated with ixekizumab.

First, genomic profiling was performed on normal CD14+ monocytes treated with IL-17A (200 ng ml^{-1}) for 24 hours in culture. Genes that were induced included pro-inflammatory cytokines (>1.5-fold, $P<0.1$), chemokines, genes associated with inflammatory response, and molecules involved in cytokine signal transduction (Supplementary Table S1a and b online). We also observed upregulation of chemokine receptors, which mediate monocyte chemotaxis (Supplementary Table S1c online), such as CXCR4 and CCR2, as well as cell adhesion molecules that mediate the rolling and docking of monocytes on vascular endothelial cells, including integrin α4, integrin β2, platelet/endothelial cell adhesion molecule 1, and CD99, which are known to be critical for plaque formation in atherosclerosis (Supplementary Table S1d online). These findings were confirmed with Ingenuity Pathway Analysis software, which identified signaling pathways that were most significantly affected in monocytes after IL-17 stimulation. The Ingenuity Pathway Analysis system identified numerous pathways associated with a pro-inflammatory response that were positively enriched, such as IL-17 signaling, IL-6/STAT3 signaling, as well as the leukocyte transendothelial migration pathway, which includes additional cell adhesion molecules associated with platelet accumulation in atherosclerosis (Figure 1).

Figure 1. Ingenuity pathway analysis (IPA) software was used to identify altered gene expression in proinflammatory signaling pathways. (**a**–**c**) Left panels: transcriptional changes in monocytes treated with IL-17 (200 ng ml^{-1}) for 24 hours vs. control. (**d**–**f**) Right panels: transcriptional changes in the blood of psoriasis patients after ixekizumab treatment (150 mg week 2 vs. baseline). Gene expression networks that are displayed include (**a**, **d**) the IL-17 signaling pathway, (**b**, **e**) the IL-6/STAT3 signaling pathway, and (**c**, **f**) leukocyte transendothelial migration. Green designates inhibitions in gene expression; red designates increases in gene expression. Applies to **a**–**f**.

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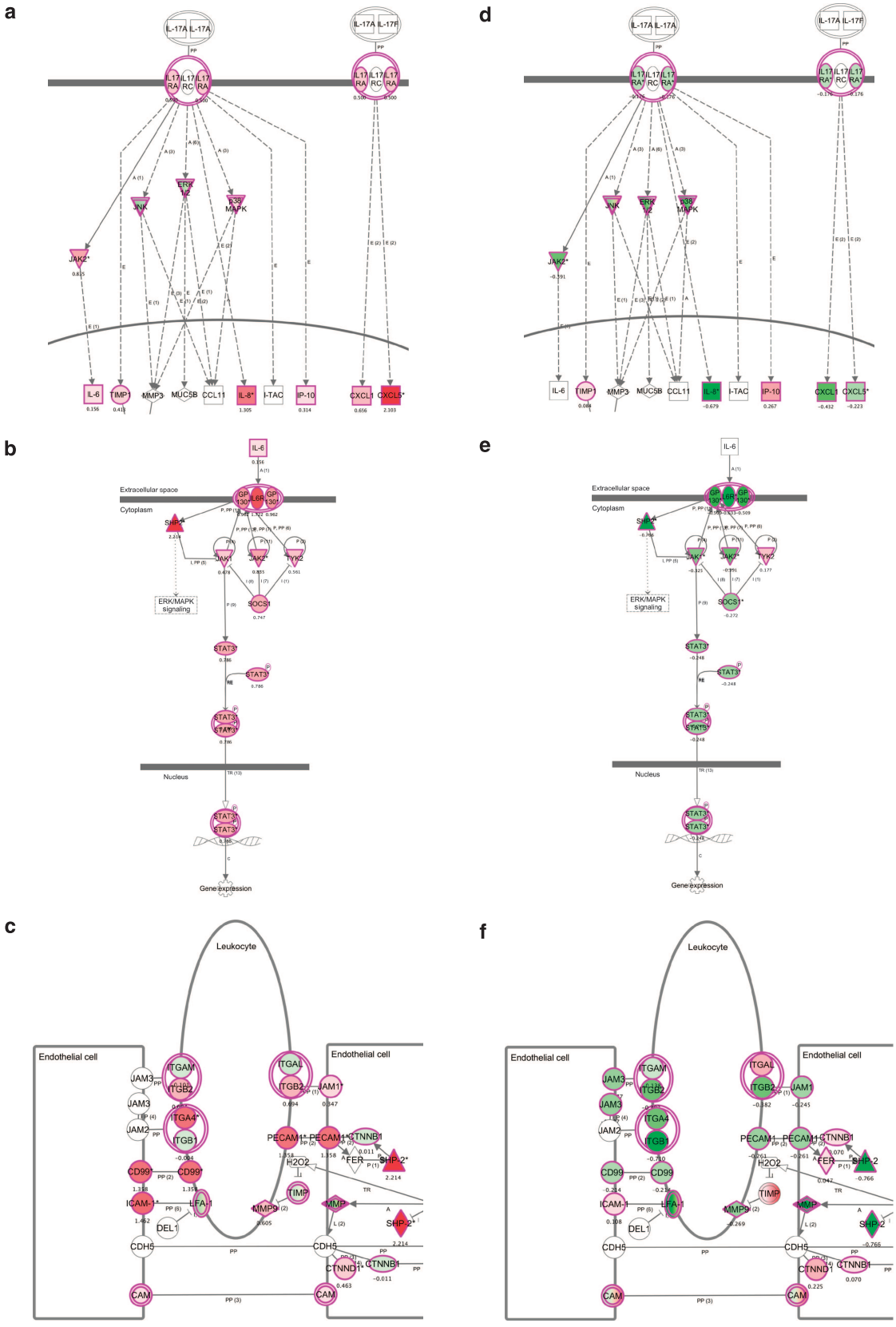
a
IL-17A IL-17A
IL-17A IL-17F
IL-6
TIMP1
MMP3
MUC5B
CCL11
IL-8*
I-TAC
IP-10
CXCL1
CXCL5*
d
b
Extracellular space
Cytoplasm
ERK/MAPK signaling
Nucleus
Gene expression
e
c
Leukocyte
Endothelial cell
f

In the second part of our analysis, we explored the impact of systemic neutralization of IL-17 on the expression of genes associated with both immune activation and atherosclerosis in blood samples from psoriasis patients treated with ixekizumab (150 mg at week 2 vs. baseline). In comparison with the observations from the *in vitro* experiments, systemic neutralization of IL-17 reduced the expression of pro-inflammatory cytokines, chemokines, and their receptors, as well as genes involved in inflammatory response. Notably, there was reduced expression of cell surface molecules that mediate leukocyte extravasation (Supplementary Table S2c online), especially those that mediate interactions of monocytes with the vascular endothelium, including matrix metalloproteinases, small GTPase Cdc42, and integrins α4 and β1 (Supplementary Table S2c and d online). Again, this blood-derived transcriptome was overlapped with the Ingenuity Pathway Analysis system, and in comparison with the pathway changes we observed in cultured monocytes, IL-17 signaling, IL-6/STAT3 signaling, and leukocyte transendothelial migration were significantly repressed for nearly all gene members after ixekizumab treatment (Figure 1).

To further explore the role of IL-17 in systemic inflammation in psoriasis patient blood, we employed gene set enrichment analysis. In patients treated with ixekizumab compared with baseline, there was broad suppression of genes that belonged to pathways that regulate inflammatory responses, leukocyte transendothelial migration, monocyte activities, and blood coagulation each with a normalized enrichment score < -1.4 (Supplementary Figure S1 online). In comparison, these same pathways were broadly upregulated in the blood-derived transcriptome of psoriasis patients compared with healthy volunteers with a normalized enrichment score of $> +1.7$ for each gene set (Supplementary Figure S1 online). In addition, gene set enrichment analysis showed significant suppression of atherosclerosis signaling in the blood transcriptome of psoriasis patients after ixekizumab treatment ($P<0.05$, false discovery rate $q<0.1$) with a normalized enrichment score of -1.5 (Supplementary Figure S2 online). Among the most suppressed genes were *ALOX5AP* (-1.4-fold decrease, $P<10^{-3}$) and *ALOX5*, which have been implicated in the inflammation of the arterial wall and have been associated with increased risk for atherosclerosis (Back, 2009). The expression of thrombospondin-1, which is involved in the formation of atherosclerotic lesions (Moura *et al.*, 2008), was decreased by 1.56-fold ($P<0.05$), and genes related to atherosclerosis disease risks and platelet aggregation (e.g., *F2RL2*) were also suppressed.

Cardiovascular disease is one of the most prevalent comorbidities associated with psoriasis (Mehta *et al.*, 2010) and contributes substantially to the morbidity and mortality rates in psoriatic patients (Pietrzak *et al.*, 2013). Recent studies have indicated that IL-17 may be associated with the development of cardiovascular disease and atherosclerosis (Ding *et al.*, 2011; Madhur *et al.*, 2011). In the apoE-deficient mouse model, blockade of IL-17 effectively reduced atherosclerosis in mice (Smith *et al.*, 2010). Furthermore, the monocyte-macrophage has an important role in the development of atherosclerosis.

Using monocytes from healthy individuals, we generated a baseline response of monocytes to IL-17. Conceivably, monocytes from psoriasis patients may behave differently *in vitro*, as they are already exposed to high levels of IL-17 in circulation (Suárez-Fariñas *et al.*, 2012) and are known to overproduce cytokines (e.g., IL-1β, IL-6, IL-8 and tumor necrosis factor) (Mizutani *et al.*, 1997; Okubo and Koga, 1998). Our data showed that IL-17 promotes an inflammatory state in blood monocytes. Remarkably, many of the induced genes are associated with cardiovascular disease risk, including IL-8, CXCL1, CXCL5, and IL-1α/β (Holm *et al.*, 2003; Rousselle *et al.*, 2013). In addition, CCR2/CCL2 and CXCR4/SDF-1 are receptor/ligand pairs that have been considered as therapeutic targets for atherosclerosis (Boring *et al.*, 1998; Wen *et al.*, 2012); IL-17 may also promote monocyte adhesion by inducing molecules (e.g., ICAM1, integrin α4, platelet/endothelial cell adhesion molecule 1, CD99) that are essential for transient interactions between leukocytes and the blood vessel wall (Woollard and Geissmann, 2010).

Analysis of the blood transcriptome in psoriasis patients receiving ixekizumab revealed decreased expression of chemokines and receptors that are associated with atherosclerosis disease risk, including CXCR4 and CXCR6. Other factors, such as GM-CSF, trigger the differentiation of monocytes into subpopulations of macrophages at atherosclerotic lesions (Di Gregoli and Johnson, 2012). ALOX5AP is required for leukotriene synthesis, and inhibitors of the 5-lipoxygenase pathways are being tested for cardiovascular disease in clinical trials (Back, 2009). As CD14+ monocytes represent only about 10% of all blood leukocytes in humans, decreased inflammation in the ixekizumab-treated blood transcriptome may reflect changes in the inflammatory states of other leukocyte populations, which warrants further investigation.

Past studies in rheumatoid arthritis have shown that systemic immune inhibitors can prevent atherosclerosis and lower the risk for cardiovascular disease (Pietrzak *et al.*, 2013). For instance, tumor necrosis factor blockers, including infliximab, etanercept, and adalimumab, have been found to delay the first cardiovascular event and reduce the incidence of myocardial infarction and cardiovascular-related mortality in rheumatoid arthritis patients (Jacobsson *et al.*, 2005; Dixon *et al.*, 2007; Kerekes *et al.*, 2009). In psoriasis patients, antitumor necrosis factor treatment was associated with decreased levels of serum inflammatory markers related to cardiovascular risks such as C-reactive protein, homocysteine, and other proinflammatory cytokines (Mastroianni *et al.*, 2005; Strober *et al.*, 2008; Yost and Gudjonsson, 2009). However, the impact of systemic anti-inflammatory therapies on cardiovascular disease outcomes has yet to be established, and other retrospective studies have reported inconsistent findings. A recent Danish study using nationwide prospectively recorded registries showed significantly decreased rates of cardiovascular disease events in patients treated with biological agents and methotrexate compared with those treated with other anti-psoriatic therapies (Ahlehoff

et al., 2013). In another report, the protective effects of systemic anti-inflammatory treatment on myocardial infarction were only found in younger patients but not in the general population (Abuabara *et al.*, 2011). In addition, increased occurrence of cardiovascular disease events has been reported in randomized controlled clinical trials of anti-IL-12/23 therapies (Tzellos *et al.*, 2013), but a meta-analysis of long-term (5 years) safety shows no increased risk for cardiovascular disease events during ustekinumab treatment (Papp *et al.*, 2013). Therefore, prospective studies with accepted surrogate end points or adverse cardiovascular event rates are needed to determine the impact of anti-IL-17 treatment for cardiovascular disease.

Overall, our data suggest that IL-17 has an important role in establishing systemic inflammation in psoriasis and may do so via its effects on monocytes. Our findings will be important for understanding how neutralization of IL-17 might attenuate systemic inflammation and impact psoriasis-associated comorbidities such as cardiovascular disease and atherosclerosis.

The clinical trial of ixekizumab in patients with psoriasis was conducted according to the principles expressed in the Declaration of Helsinki, and written informed consent was obtained from all subjects, who provided samples in this study.

CONFLICT OF INTEREST

JGK has received research funds from Eli Lilly and Co. RWH, ED, DS, and MPH are all employees and stockholders at Eli Lilly and Co. CQFW, KN, and CM state no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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